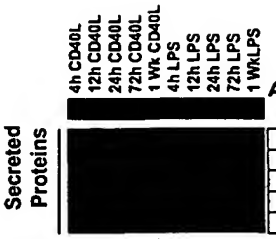
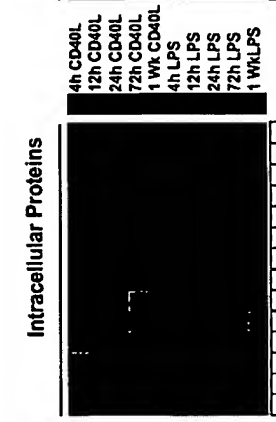
A



Accession Number	Gene	Description
W62918	EBI3	Epstein-Barr Virus-induced gene 3 (IL-12 p40 related)
X01971	IFNα5	Interferona Family Gene 5
U02298	RANTES	Chemokine CCL5
X56602	ISG15	Interferon-stimulated Gene 15kD
M86829	IP10	Chemokine CXCL10



Accession Number	Gene	Description
L32974	IFIT3	Interferon-Induced Protein w. Tetratricopeptide Repeats
M21117	Mx1	Interferon-Induced GTPase
U43085	IFIT2	Interferon-Induced Protein w. Tetratricopeptide Repeats
U43084	IFIT1	Interferon-Induced Protein w. Tetratricopeptide Repeats
U73037	IRF7	Interferon Regulatory Factor 7
AA146282	Similar to IFI204	Similar to Interferon-Activable Protein 204
AA013783	Similar to hIFI35	Similar to Human Interferon-Induced 35kD Protein
AA163988	Similar to IFI203	Similar to Interferon-Activable Protein 203
L38281	IRG1	Immune-Responsive Gene 1
U51992	IRF9	Interferon Regulatory Factor 9
U19119	IFI1	Interferon-Inducible Protein 1
AA034646	Cbkr1l2	Chemokine (C-C) Receptor 1, -like 2
W29434	IFI-TM3l	Interferon-Induced Transmembrane Protein 3-like
U09928	PKR	dsRNA-Activated Protein Kinase

B

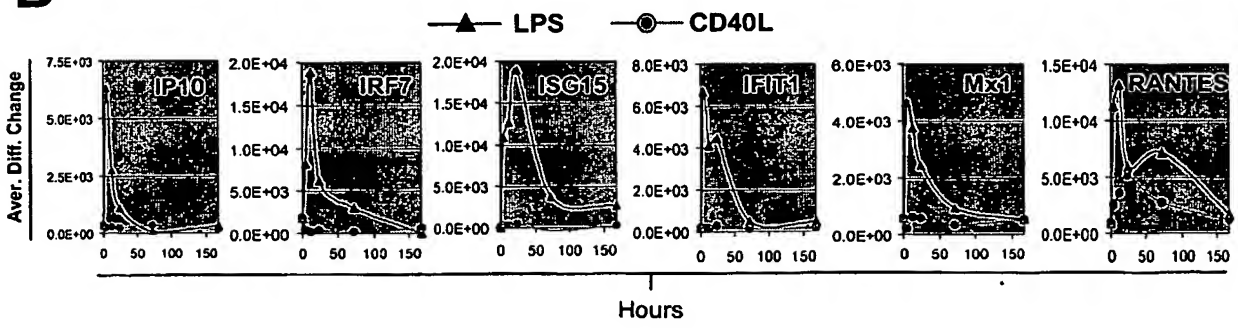
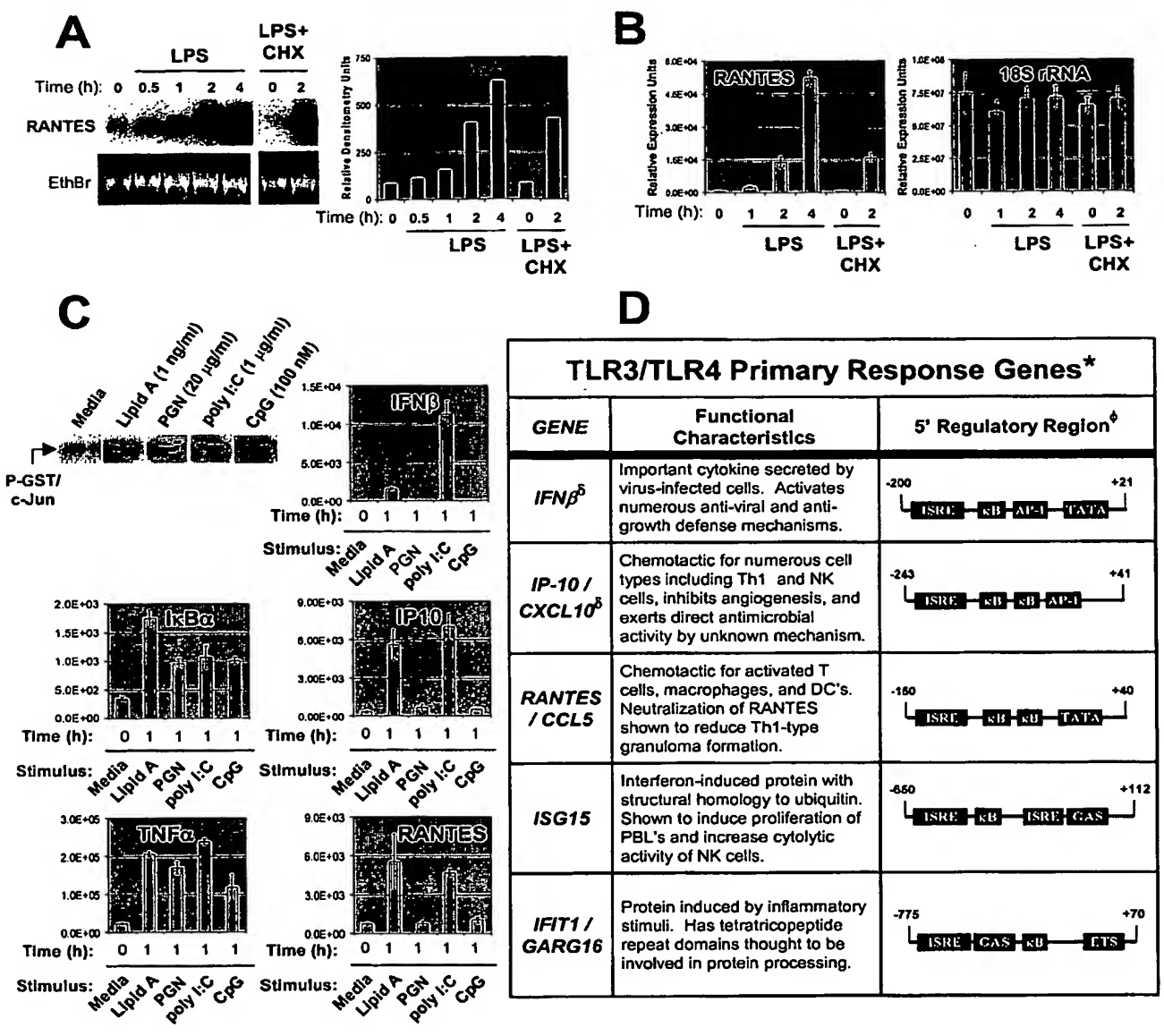


FIGURE 1



TLR3/TLR4 Primary Response Genes*

GENE	Functional Characteristics	5' Regulatory Region$^{\phi}$
IFNβ$^{\delta}$	Important cytokine secreted by virus-infected cells. Activates numerous anti-viral and anti-growth defense mechanisms.	-200 ISRE κB AP-1 TATA +21
IP-10 / CXCL10$^{\delta}$	Chemotactic for numerous cell types including Th1 and NK cells, inhibits angiogenesis, and exerts direct antimicrobial activity by unknown mechanism.	-243 ISRE κB κB AP-1 +41
RANTES / CCL5	Chemotactic for activated T cells, macrophages, and DC's. Neutralization of RANTES shown to reduce Th1-type granuloma formation.	-150 ISRE κB κB TATA +40
ISG15	Interferon-induced protein with structural homology to ubiquitin. Shown to induce proliferation of PBL's and increase cytolytic activity of NK cells.	-650 ISRE κB ISRE GAS +112
IFIT1 / GARG16	Protein induced by inflammatory stimuli. Has tetratricopeptide repeat domains thought to be involved in protein processing.	-775 ISRE GAS κB ETS +70

FIGURE 2

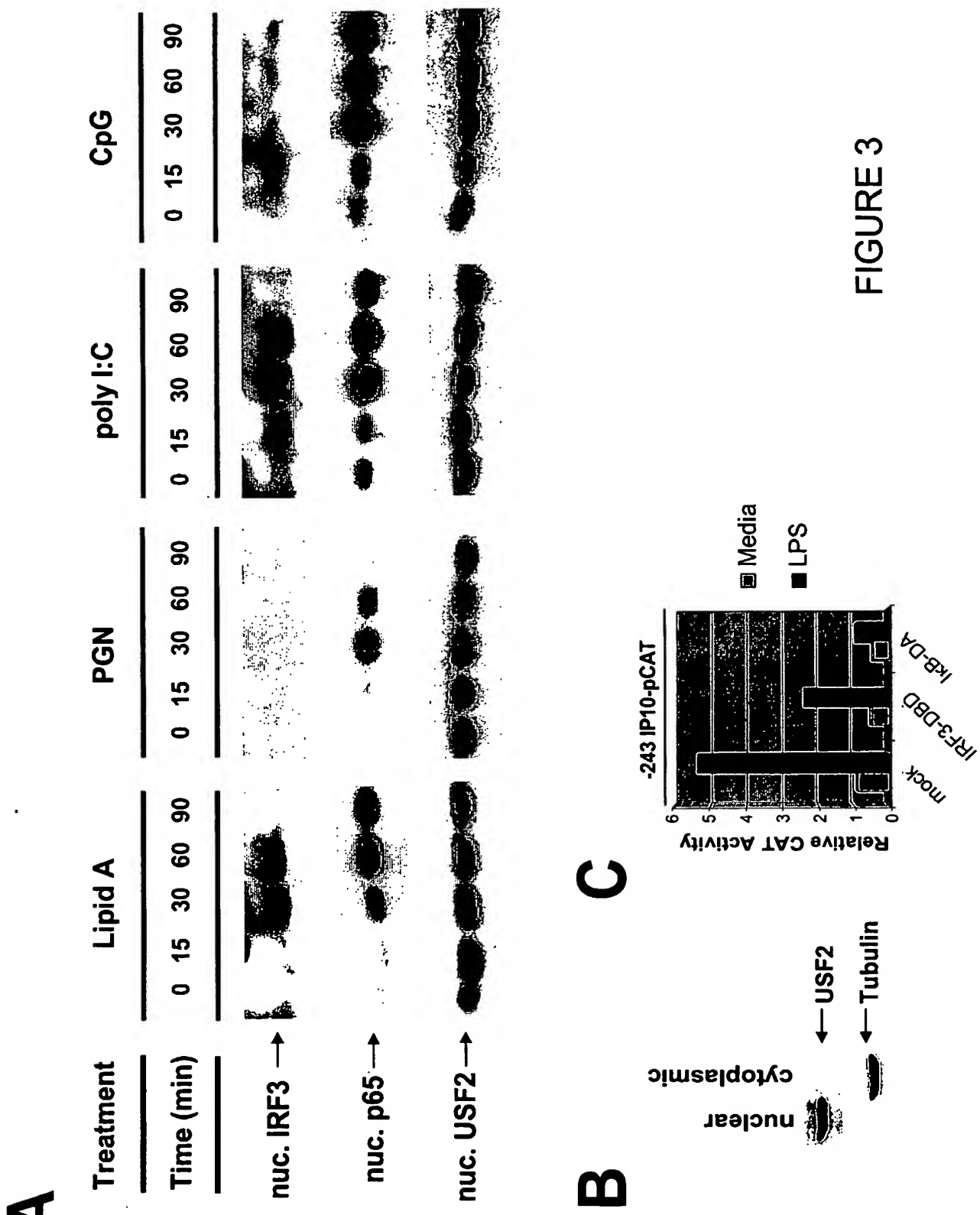

A
Treatment
Lipid A
PGN
poly I:C
CpG
Time (min)
0 15 30 60 90
nuc. IRF3
nuc. p65
nuc. USF2
B
nuclear
cytoplasmic
USF2
Tubulin
C
-243 IP10-pCAT
Media
LPS
Relative CAT Activity
mock
IRF3-DBD
IκB-DA

FIGURE 3

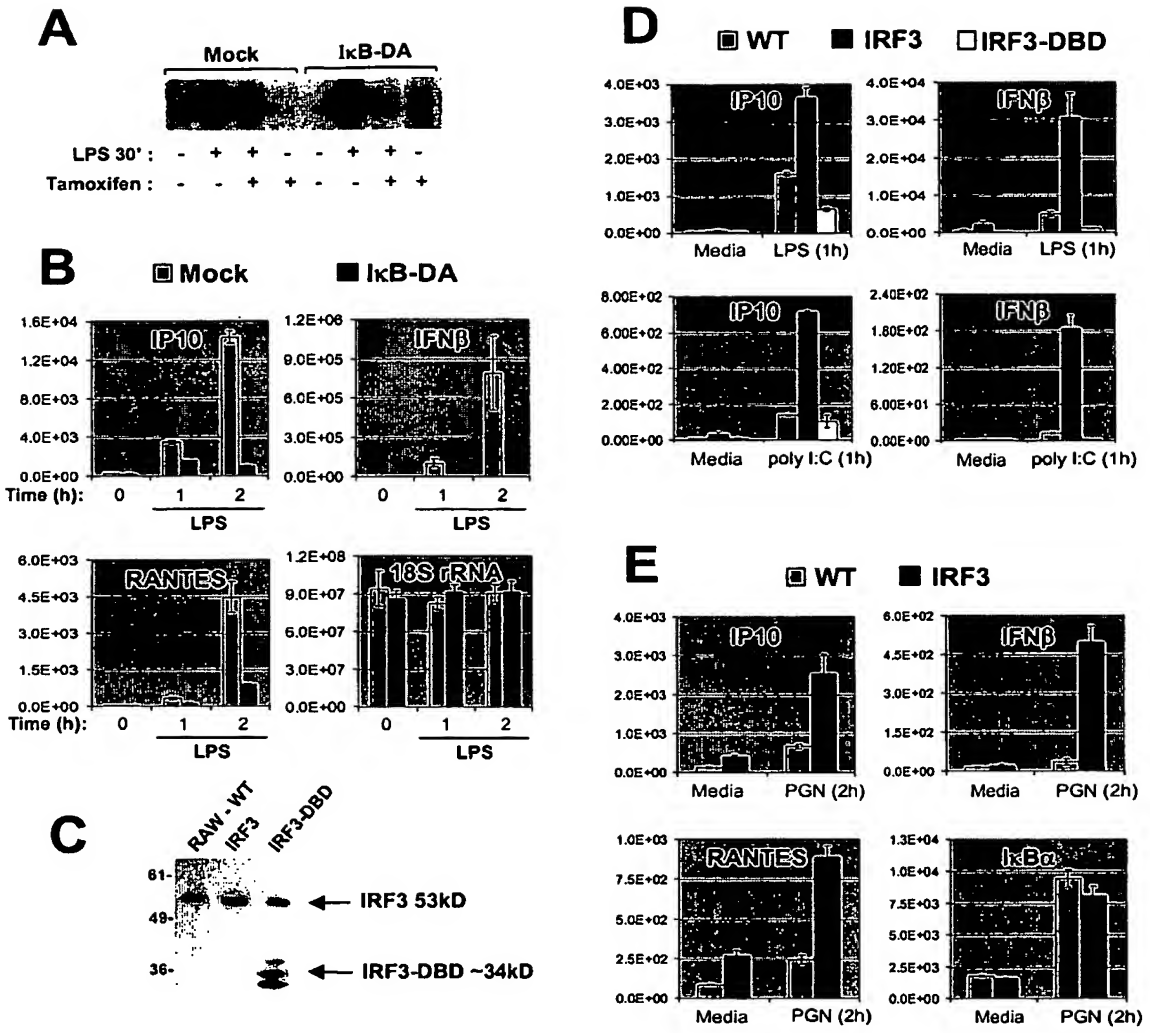
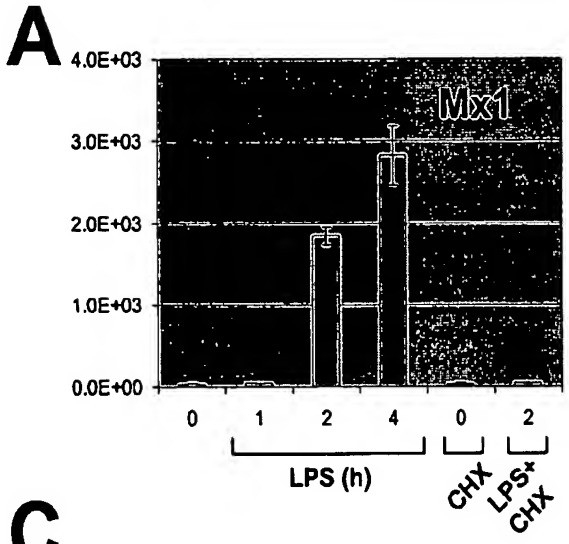
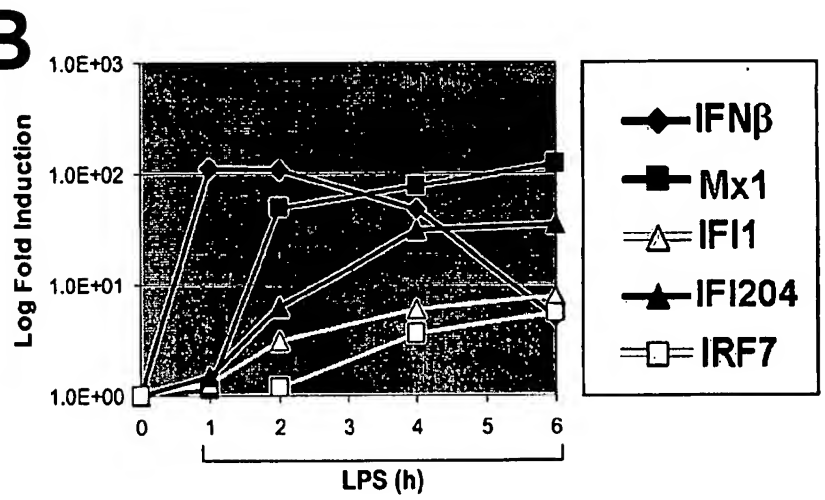

A
Mock
IκB-DA
LPS 30' : - + + - - + + -
Tamoxifen : - - + + - - + +
B
Mock
IκB-DA
IP10
IFNβ
RANTES
18S rRNA
Time (h): 0 1 2
LPS
C
RAW - WT
IRF3
IRF3-DBD
61-
49-
36-
IRF3 53kD
IRF3-DBD ~34kD
D
WT
IRF3
IRF3-DBD
IP10
IFNβ
Media
LPS (1h)
poly I:C (1h)
E
WT
IRF3
IP10
IFNβ
RANTES
IκBα
Media
PGN (2h)

FIGURE 4





C

TLR3/TLR4 Secondary Response Genes*					
GENE	Functional Characteristics	Induced by		Confers Resistance to ϕ:	
		LPS	Virus	Bacteria	Virus
Mx1	Cytoplasmic and nuclear GTPase. Resistance to virus thought to be due to indirect modification of cellular functions needed along the viral replication pathway.	+	+	N/D	+
IFI1	GTPase with unknown cellular function.	+	+	+	N/D
IFI204	Nucleolar member of HIN200 Family. Shown to inhibit growth and delay cell cycle progression; possibly by binding to Rb and UBF1.	+	+	N/D	N/D
IRF7	Member of Interferon Regulatory Factor Family. IRF3 and IRF7 shown to be required for positive feedback upregulation of IFN-α/β upon viral infection.	+	+	N/D	N/D

FIGURE 5

10/512124
PCT/US03/12751

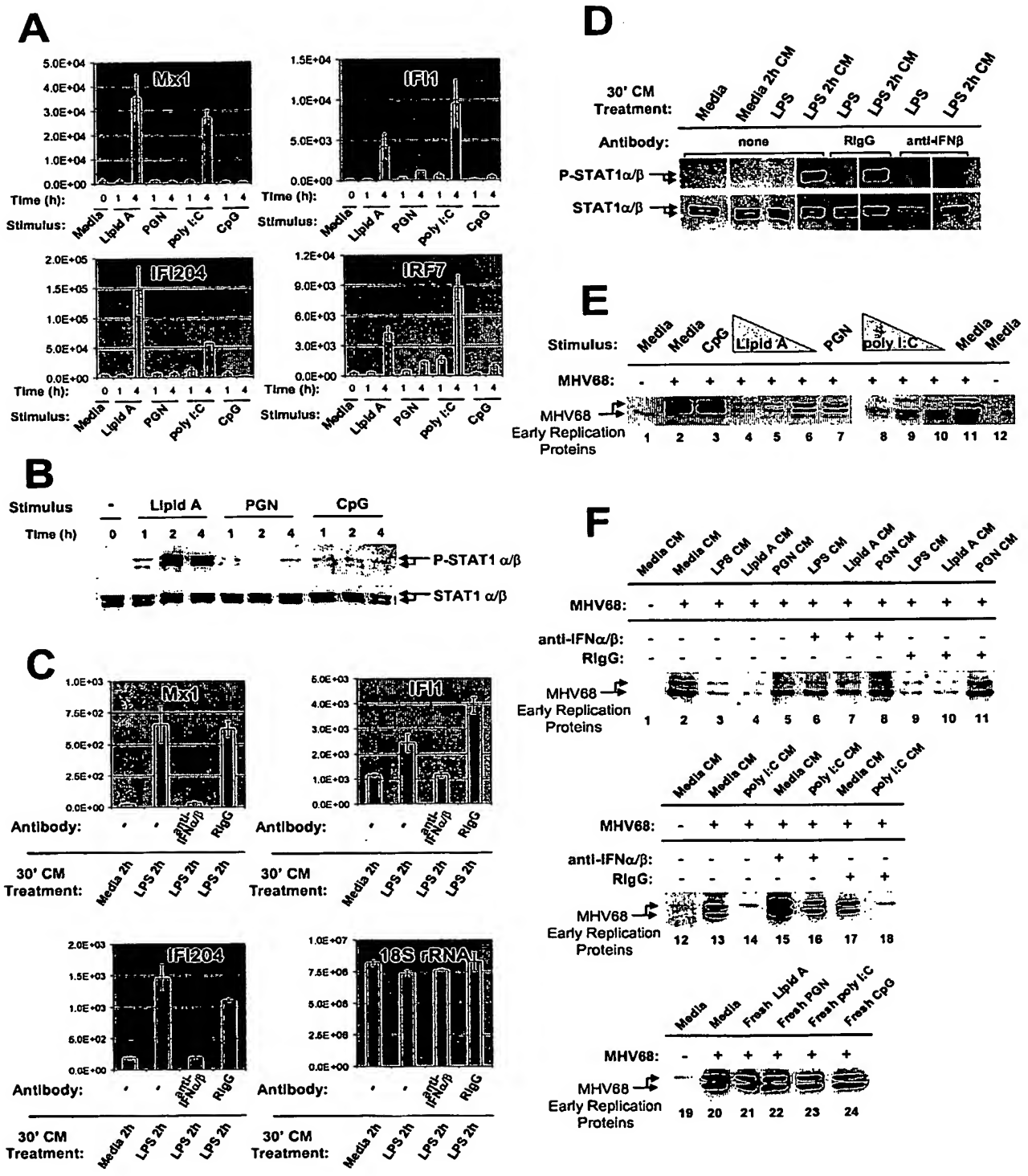

A
Mx1
IFI1
IFI204
IRF7
Time (h):
Stimulus:
Media
Lipid A
PGN
Poly I:C
CpG
B
Stimulus
Time (h)
P-STAT1 α/β
STAT1 α/β
C
Antibody:
RIgG
30' CM Treatment:
Media 2h
LPS 2h
18S rRNA
D
30' CM Treatment:
Media 2h CM
LPS 2h CM
Antibody:
none
RIgG
anti-IFNβ
P-STAT1α/β
STAT1α/β
E
Stimulus:
MHV68:
MHV68 Early Replication Proteins
F
Media CM
LPS CM
Lipid A CM
PGN CM
poly I:C CM
anti-IFNα/β:
RIgG:
Fresh Lipid A
Fresh PGN
Fresh poly I:C
Fresh CpG

FIGURE 6

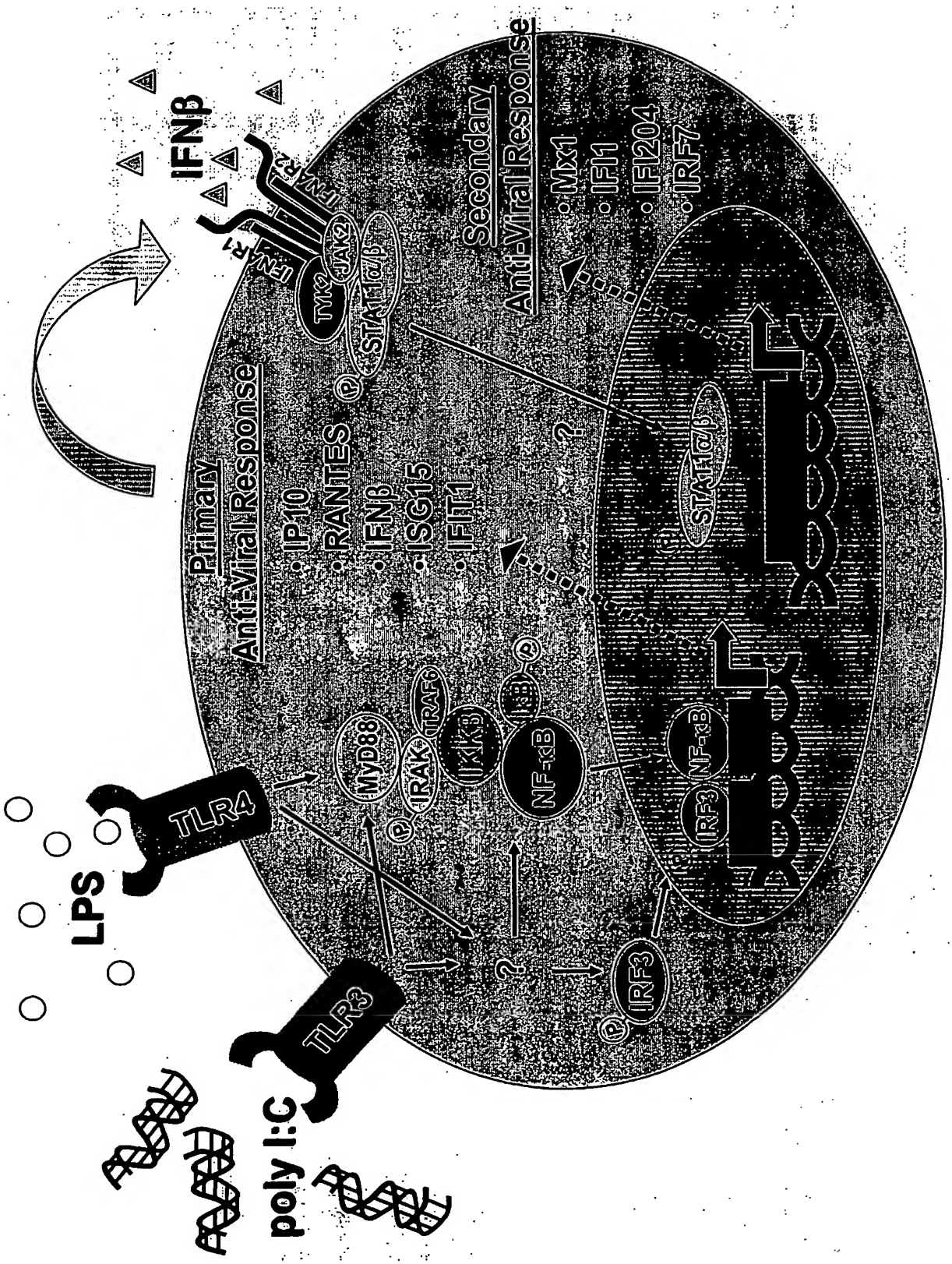

LPS
poly I:C
TLR4
TLR3
MyD88
IRAK
TRAF6
IKK
IκB
NF-κB
IRF3
IFNβ
IFNAR1
IFNAR2
TYK2
JAK2
STAT1α/β
Primary
Anti-Viral Response
IP10
RANTES
IFNβ
ISG15
IFIT1
Secondary
Anti-Viral Response
Mx1
IFI1
IFI204
IRF7

FIGURE 7

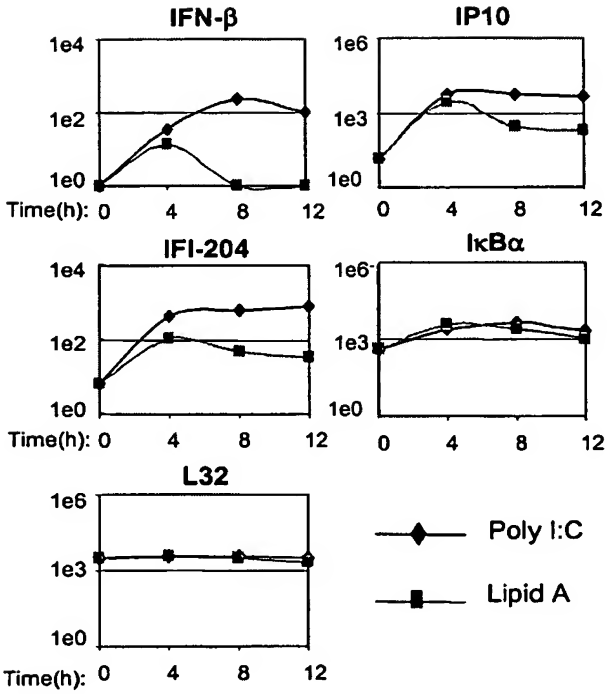

IFN-β
IP10
IFI-204
IκBα
L32
1e4
1e2
1e0
1e6
1e3
Time(h): 0 4 8 12
Poly I:C
Lipid A

FIGURE 8

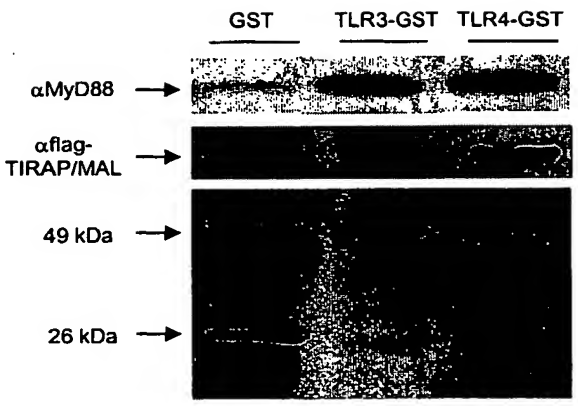


FIGURE 9

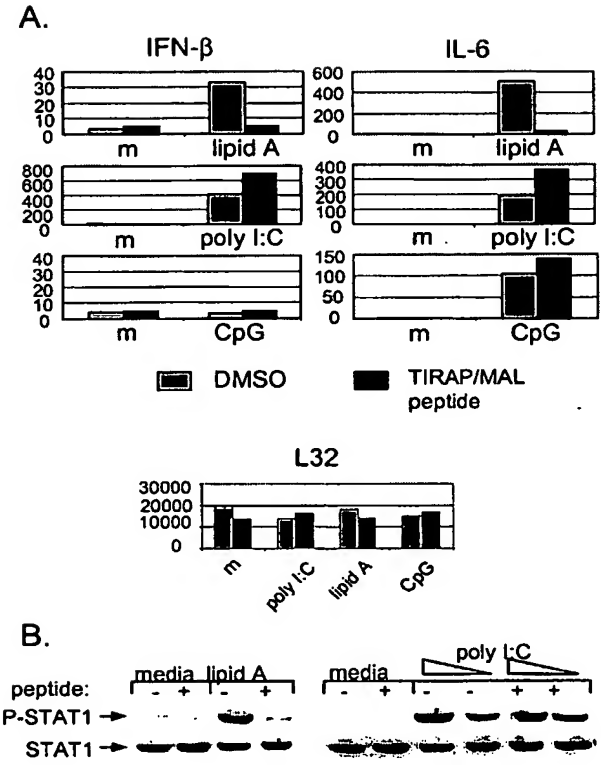

A.
IFN-β
IL-6
m
lipid A
poly I:C
CpG
DMSO
TIRAP/MAL peptide
L32
B.
media
lipid A
poly I:C
peptide:
P-STAT1
STAT1

FIGURE 10

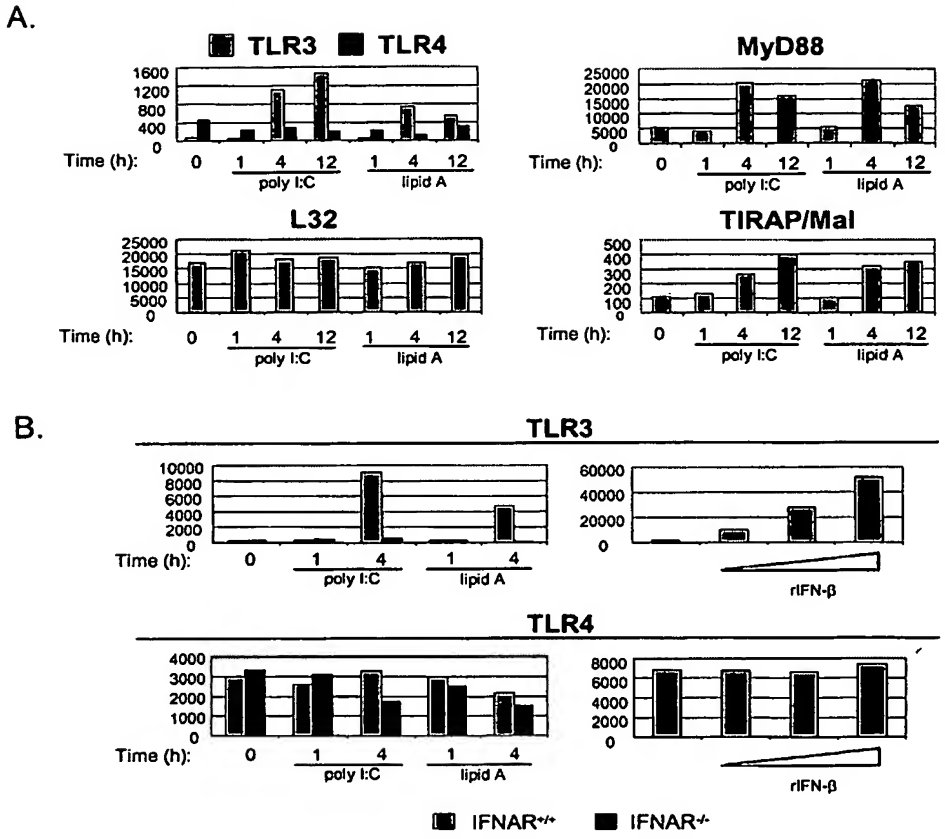

A.
TLR3
TLR4
MyD88
L32
TIRAP/Mal
Time (h):
0 1 4 12 1 4 12
poly I:C
lipid A
B.
TLR3
TLR4
rIFN-β
IFNAR+/+
IFNAR-/-

FIGURE 11

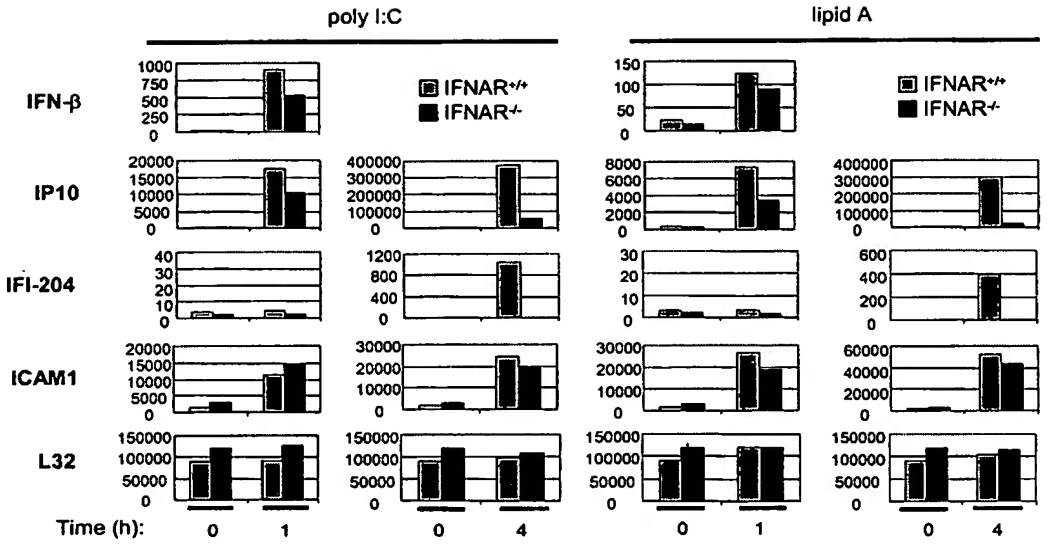

poly I:C
lipid A
IFNAR+/+
IFNAR-/-
IFN-β
IP10
IFI-204
ICAM1
L32
Time (h):
0
1
0
4
0
1
0
4

FIGURE 12

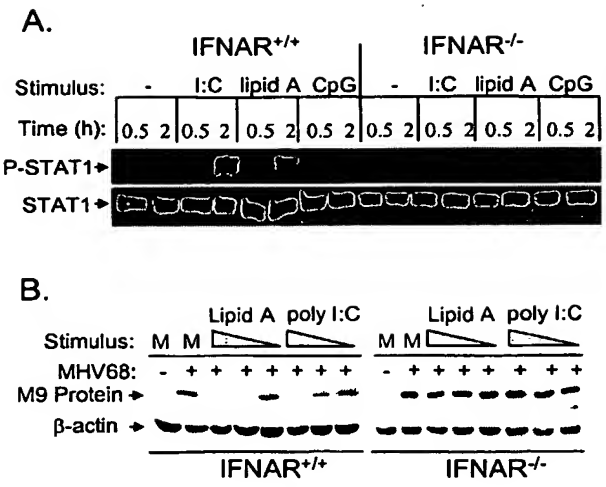
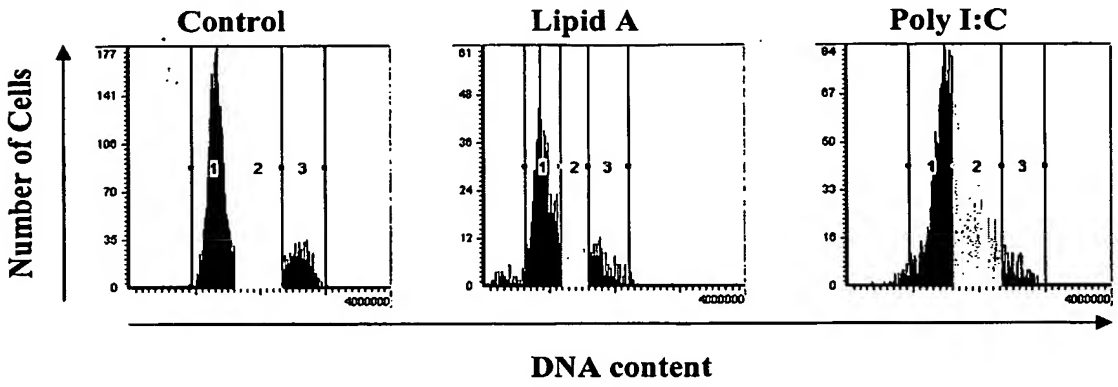

A.
IFNAR+/+
IFNAR-/-
Stimulus: - I:C lipid A CpG - I:C lipid A CpG
Time (h): 0.5 2 0.5 2 0.5 2 0.5 2 0.5 2 0.5 2 0.5 2 0.5 2
P-STAT1
STAT1
B.
Lipid A poly I:C Lipid A poly I:C
Stimulus: M M M M
MHV68: - + + + + + + + - + + + + + + +
M9 Protein
β-actin
IFNAR+/+
IFNAR-/-

FIGURE 13

TLR3/4 Activation Leads to an IFN-dependent G1/S Block in Murine Macrophage Cells

A.



B.

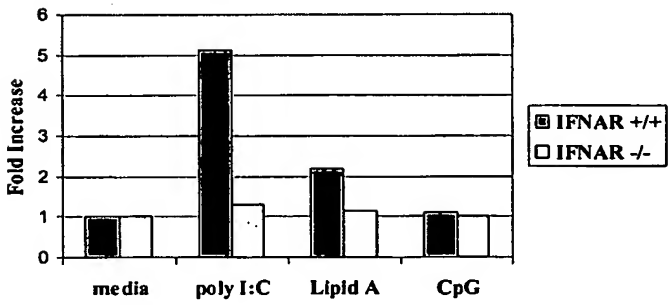
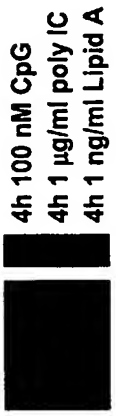


FIGURE 14

TLR3/4 Specificity Upregulate Genes Involved in the G1/S Transition

A.



Accession Number	Description
M96163	Serum-Inducible Kinase (SNK)
AF005885	Cyclin G2
W90992	Cyclin D2
M83749	Cyclin D2

B.

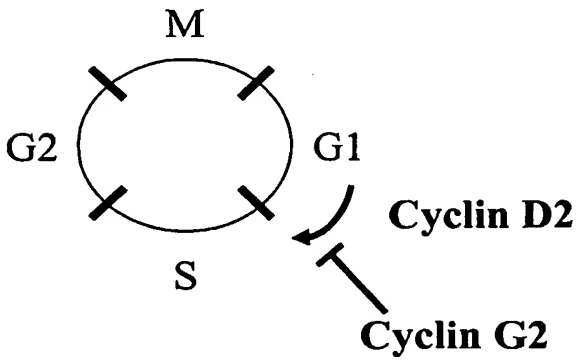


FIGURE 15

TLR3 Activation Increases Apoptosis in the RAW 264.7 Macrophage Cell Line

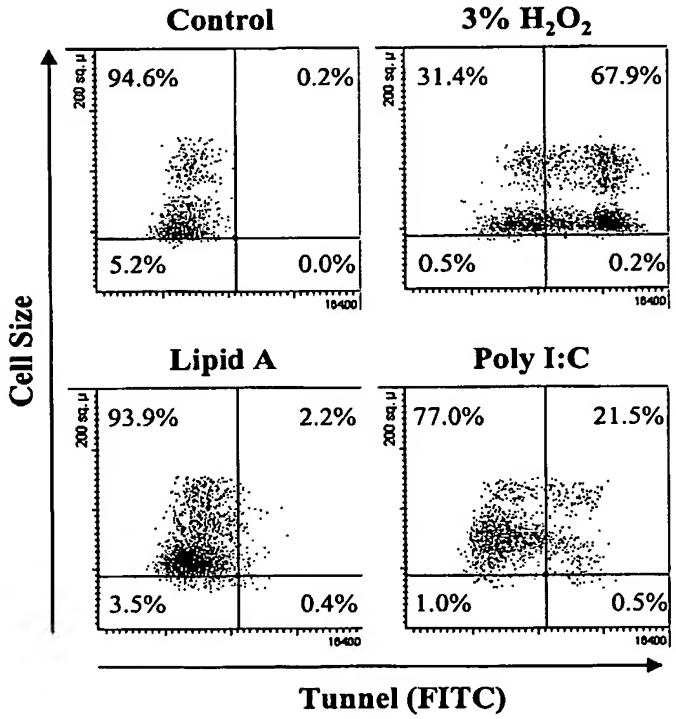


FIGURE 16

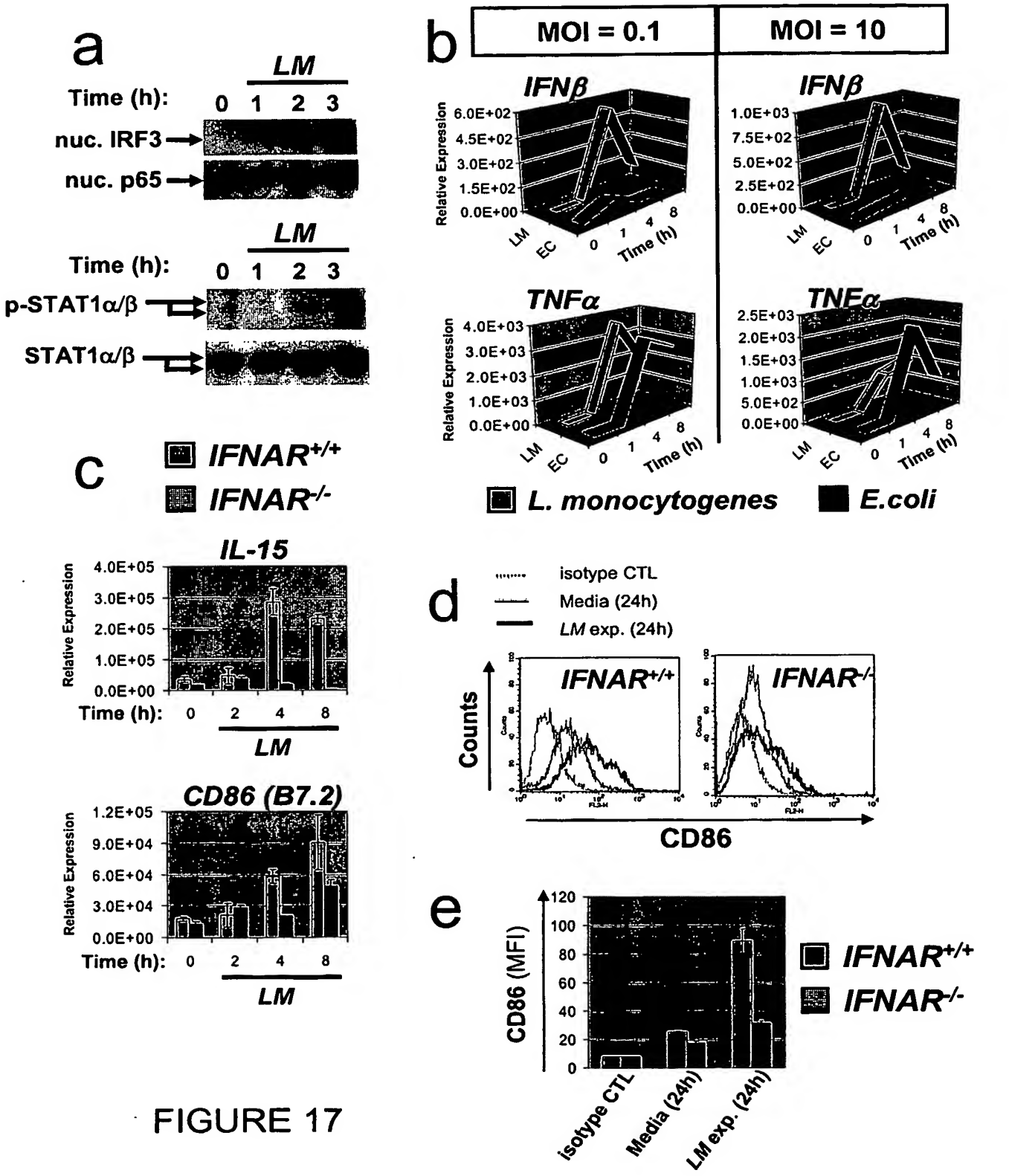

a
LM
Time (h): 0 1 2 3
nuc. IRF3
nuc. p65
LM
Time (h): 0 1 2 3
p-STAT1α/β
STAT1α/β
b
MOI = 0.1
MOI = 10
IFNβ
TNFα
Relative Expression
LM
EC
Time (h)
L. monocytogenes
E.coli
c
IFNAR+/+
IFNAR-/-
IL-15
CD86 (B7.2)
Relative Expression
Time (h): 0 2 4 8
LM
d
isotype CTL
Media (24h)
LM exp. (24h)
IFNAR+/+
IFNAR-/-
Counts
CD86
e
CD86 (MFI)
isotype CTL
Media (24h)
LM exp. (24h)
IFNAR+/+
IFNAR-/-

FIGURE 17